Addis Ababa Science and Technology university Collage of Architecture and Civil Engineering

Department; Geotechnical Engineering

EVALUATION OF MICROBIAL INDUCED CALCITE PRECIPITATION METHOD AS A SOIL STABILIZATION

By: BELEN TESFAHUN
Advisor Name:

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CERTIFICATION

The Project titled " EVALUATION OF MICROBIAL INDUCED CALCITE PRECIPITATION METHOD AS A SOIL STABILIZATION" by Belen Tesfahun meets the regulations governing the award of the degree of Master of Engineering (M.Eng) in Geotechnical Engineering in Addis Ababa Science and Technology University and is approved for its contribution to knowledge and literary presentation.

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CC	Calcium Chloride
CIPS	Calcite in-situ precipitation system
DIC	Concentration of dissolved inorganic carbon
ES	Egg Shell
MICP	Microbial induced Calcite precipitation
MIC	Microbial induced calcium
MSE	Mechanical stabilization of earth
OD	Optical density
SEM	Scanning electron microscope
UCS	Unconfined compressive strength
USCS	Unified soil classification system

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ABSTRACT

A newly emerging microbiological soil stabilization method, known as microbial induced calcite precipitation (MICP), is tested for geotechnical engineering applications. MICP is a promising technique that utilizes the metabolic pathways of bacteria to form calcite precipitation throughout soil matrix, to improve engineering properties of soil through formations of coating and bonds between soil particles. Strength and permeability of a given soil samples has been evaluated based on three researcher's laboratory test results on UCS & permeability. During the evaluation each researcher's individual test result on UCS and permeability in relation to calcium carbonate precipitation ($CaCO_3$) has been evaluated. Calcium carbonate precipitation is the result of microbial processes in which the bacteria produce during the metabolism process, this $CaCO_3$ content will bind the soil particle which will reduce the permeability of the soil and also increase the unconfined compression strength of microbial treated soil. From the evaluation it shows an improvement of the permeability and strength of the tested soils. The result show an increase in $CaCO_3$ content in the soil results in an increment of strength and reduction of permeability. The project gave an insight about MICP soil stabilization method effectiveness on improving the shear strength and permeability of a given soil sample but it needs a detailed study on to what extent it improves the shear strength and permeability of a given soil sample.

Keywords: MICP, soil stabilizations, Unconfined compressive strength, Permeability.

1. CHAPTER ONE: INTRODUCTION

1.1 BACKGROUND

There are extensive areas of ground where mining and excavations for coal, chalk, sand and gravel may have made the ground unstable. Where such works have previously existed the ground and building foundations may well be subject to periodic, unpredictable subsidence. Specialist advice should always be sought to establish the extent of previous workings and the most appropriate method of designing foundations for such situations (Atticus, 2012)

Where it is known that ground may be unstable and there is no ready means of predicting the possibility of mass movement of the subsoil and it is expedient to build, a solution is to use some form of reinforced concrete raft under the whole of the buildings, The concrete raft, which is cast on or just below the surface, is designed to spread the load of the building over the whole of the underside of the raft so that, in a sense, the raft floats on the surface. Alternatively, if there is good load bearing strata, but they are some meters below the surface, pile foundations can be used. Traditional concrete foundations may be reinforced with the use of steel frames or other types of composite materials to increase tensile strength of the foundation. However, these alterations may not help stabilize the foundation work and prevent cracking if not complete collapsing of columns supporting the building structures. These kinds of defects may not be easily identified or corrected satisfactorily. With a growing number of alternative approaches to construction inspired by more sustainable architecture and also advances in prefabrication, some alternative approaches to traditional concrete foundations are being used

In recent times advances have been made in the field of geotechnical engineering where by soil treatment and improvement works would rather provide preferred options for stabilizing the underneath soil over which a foundation is to be laid . Soil stabilization requires improvement of one or more of the soil behaviors such as tensile strength, porosity or compactness etc. However, to bring such improvement in the soil behaviors, a sample of soil should be subjected to a series of physico-chemical experimental procedures to bring about significant changes or alterations in certain chemical or physical behavior of the foundation soil. Indeed, soil stabilization is a process of altering some of its behaviors and makes it suitable for a foundation work. In broader sense, it is a general term for any physical, chemical, biological or combined method of changing a natural soil to meet an engineering purpose (Atticus, 2012).

Bio-mediated method of soil improvement generally refers to the biochemical reaction that takes place within a soil mass to produce calcite precipitate to modify some engineering properties of the soil meanwhile, utilizing the interdisciplinary knowledge of civil engineering, chemistry and microbiology to alter the soil engineering properties in the subsurface has emerged recently (Stocks-Fischer & Bang, 1999) The technique utilizes soil microbial processes. It was revealed that microorganisms facilitate chemical reactions within a soil mass, promote weathering and change the chemical and mechanical properties of specimens after sampling (Dejong, 2008).

Soil stabilization using microbial geo-technology is an emerging branch of Geotechnical Engineering which uses microorganisms as a soil stabilizing agent. Although there are various potential applications of microorganisms to geotechnical engineering, at the present, promising applications are only concentrated in the bio-clogging and bio-cementation. Microbial geo-technology has advantages of low investment and maintenance costs. Therefore, this review is covering mainly the recent developments in these two areas (Ivanov & J.chu, 2008).

Bio-clogging is to reduce the hydraulic conductivity of soil and porous rocks due to microbial activity or products. It could be used to reduce drain channel erosion, form grout curtains to reduce the migration of heavy metals and organic pollutants, and prevent piping of earth dams and dikes. Bio-cementation is to enhance the strength and stiffness properties of soil and rocks though microbial activity or products. It could be used to prevent soil avalanching, reduce the swelling potential of clayey soil, mitigate the liquefaction potential of sand, and compact soil on reclaimed land sites.

The major factors that affect the applications of microorganisms to geotechnical engineering include the screening and identification of suitable microorganisms for different applications and different environments, the optimization of microbial activity *in situ*, biosafety of the application, cost effectiveness, and stability of soil properties after bio-modification (Stocks-Fischer & Bang, 1999). Among all the factors, cost effectiveness is the most important factor for large-scale application. This project paper deals with the review of the available literature on the recent developments related to bio-clogging and bio-cementation with the aims to offer updates on the status of laboratory works currently undertaken elsewhere in the world by chemist, engineers and microbiologists and provide summary of the progress made so far in these two areas including highlights of their potential applications, advantages and disadvantages of these new technologies over the traditional and other alternative methods.

1.2 STATEMENT OF THE PROBLEM

One of the reasons for failure of structures like: building, road, dam etc. is due to ground condition, different kinds of soil improvement methods have been used to mitigate this problem. The common and traditional methods used to be involving either physical (mechanical) or chemical soil improvement mechanisms. Of course, some of these techniques are effective for many applications. Literature reports show that research advancement works are still progressing in these areas. However, many studies are also devoted to show that most of the applications using these traditional techniques including the chemical ones are very expensive, environmentally unfriendly and low effectiveness for improving the ground. Construction engineers, real estate developers and governments have begun to invest on research and urging experts to look for alternative cost effective, environmental friendly and effective ground improvement and soil-stabilization methods. Recently, microorganism based soil remediation (improvement) method has become a very promising research field, now-a-days calling researchers in multi-disciplinary areas for conducting integrated soil improvement works. This project is carried out to evaluate the effectiveness of microbial induced calcite precipitation method, since it is became one of soil stabilization method which is environmental friendly cost efficient and effective soil stabilization method.

1.3 OBJECTIVES OF THE PROJECT

Existing methods for improving the engineering properties of soils are diverse with respect to their final outcome. Microbial induced calcite precipitation method is one of recent soil stabilization method. Many researcher has proof the effectiveness of this method, in this project it tries to evaluate this soil stabilization method effectiveness by examining three researcher res On the basis of the background given above, the following general and specific objectives of the project can be stated.

1.3.1 GENERAL OBJECTIVE

The general objective of this project is to evaluate the effectiveness of MICP method by comparing three experimental studies results on improving unconfined compressive strength and permeability of a given soil sample.

1.3.2 SPECIFIC OBJECTIVES

To identify laboratory test results conducted on MICP regarding unconfined compressive strength & permeability.

To analyze the effectiveness of MICP technique by comparing laboratory results and to give a conclusion.

To give an insight of different laboratory results & what kind of method they follow.

To give a guidance on what kind of materials to use for conducting MICP technique.

To evaluate the effectiveness of microbial induced calcite precipitation method on improving one soil strength and permeability.

1.4 SCOPE OF THE PROJECT

This project tries to identify the effectiveness of MICP soil stabilization method. It mainly focused on the permeability and strength of a given soil sample. The project basically tries to evaluate three researchers work on this method and from that it draws a conclusion and recommendation.

1.5 METHODOLOGY

To achieve the objectives of this project, the following methodologies have been followed

Literature review on soil stabilization methods mainly focused on MICP method.

Gather three researcher laboratory test result data that will be an input for the evaluation of MICP soil stabilization method.

Based on the three researchers laboratory test result draw this project result by using chart & interpolation method

From the result found, using chart & interpolation draw a conclusion and recommendation of MICP soil stabilization method.

2 CHAPTER TWO: LITERATURE REVIEW

2.1 BUILDING FOUNDATION & GROUND WORKS

Soil is the general term for the upper layer of the Earth's surface that consists of various combinations of particles of disintegrated rock, such as gravel, sand or clay, with some organic remains of decayed vegetation generally close to the surface.

The common procedure requires that the building shall be constructed so that ground movement caused by swelling, shrinkage or freezing of the subsoil, or land-slip or subsidence, which can be reasonably foreseen, will not impair the stability of the building. The foundations of the building must be selected and designed so that they overcome the problems of ground movement. There are a number of familiar approaches to foundation construction, from strip foundations, piles and rafts all of which are constructed of concrete. Foundations can be constructed for changing some of the natural soil behaviors such as tensile strength, hardness etc. using standardized procedures so as to meet different engineering purposes.

More recent issues regarding the degradation of the environment has prompted developments in green technologies and sustainability. These issues are often motivated by the rapidly increasing global population and popularity of urban living, and engineers have been driven to alleviate many of these concerns. Engineering solutions are regularly impeded by geographical boundaries and inadequate soil conditions, which result in expensive designs and non-sustainable practices, including landfill and contamination of soils. Several soil stabilization or improvement techniques including soil replacement and preloading have been commonly practiced by most constructive engineers to achieve consolidation chemical admixture and grouting stabilization of soil foundation (Atticus, 2012).

Soil stabilization is a general term for any physical, chemical, biological or combined method of changing a natural soil to meet engineering purpose. Soil improvement methods can be classified in to physical (mechanical), chemical and biological methods

2.2 PHYSICAL/MECHANICAL SOIL IMPROVEMENT METHODS

According to (Chu & Ivanov, 2009) the conventional soil improvement methods can be classified in to the following groups.

A. **Ground improvement without admixtures in non-cohesive soils or fill materials**, this category is further divided in to five methods of application:

Dynamic compaction: Densification of granular soil by dropping a heavy weight from air onto ground.

Vibrio compaction: Densification of granular soil using a vibratory probe inserted into ground.

Explosive compaction: Shock waves and vibrations are generated by blasting to cause granular soil ground to settle through liquefaction or compaction.

Electric pulse compaction: Densification of granular soil using the shock waves and energy generated by electric pulse under ultra-high voltage.

Surface compaction (including rapid impact compaction): Compaction of fill or ground at the surface or shallow depth using a variety of compaction machines.

B. **Ground improvement without admixtures in cohesive soils,** this category is further is divided into seven methods of application.

Replacement/displacement (including load reduction using lightweight materials): Remove bad soil by excavation or displacement and replace it by good soil or rocks. Some lightweight materials may be used as backfill to reduce the load or earth pressure.

Preloading using fill (including the use of vertical drains): Fill is applied and removed to pre-consolidate compressible soil so that its compressibility will be much reduced when future loads are applied.

Preloading using vacuum (including combined fill and vacuum): Vacuum pressure of up to 90 kPa is used to pre-consolidate compressible soil so that its compressibility will be much reduced when future loads are applied.

Dynamic consolidation with enhanced drainage (including vacuum): Similar to dynamic compaction except vertical or horizontal drains (or together with vacuum) are used to dissipate pore pressures generated in soil during compaction.

Electro-osmosis or electro kinetic consolidation: DC current causes water in soil or solutions to flow from anodes to cathodes which are installed in soil.

Thermal stabilization: Change the physical or mechanical properties of soil permanently or temporarily by heating or freezing.

Hydro-blasting compaction: Collapsible soil (loess) is compacted by a combined wetting and deep explosion action along a borehole.

C. **Ground improvement with admixtures or inclusions**, this method is further divided in to eight categories.

Vibro replacement or stone columns: Hole jetted into soft, fine-grained soil and back filled with densely compacted gravel or sand to form columns.

Dynamic replacement: Aggregates are driven into soil by high energy dynamic impact to form columns. The backfill can be sand, gravel, stones or demolition debris.

Sand compaction piles: Sand is fed into ground through a casing pipe and compacted by vibration, dynamic impact, or static excitation to form columns.

Geo-textile confined columns: Sand is fed into a closed bottom geo-textile lined cylindrical hole to form a column.

Rigid inclusions: Use of piles, rigid or semi-rigid bodies or columns, premade or formed in-situ.

Geo-synthetic reinforced column or pile supported embankment: Use of piles, rigid or semi-rigid columns/inclusions and geo-synthetic girds to enhance the stability and reduce the settlement of embankments.

Other methods: Unconventional methods, such as formation of sand piles using blasting and the use of bamboo, timber and other natural products.

D. **Ground improvement with grouting type admixtures**: this method is further divided in to six categories.

Particulate grouting: Grout granular soil or cavities or fissures in soil or rock by injecting cement or other particulate grouts to either increase the strength or reduce the hydraulic conductivity.

Chemical grouting: Solutions of two or more chemicals react in soil pores to form a gel or a solid precipitate to either increase the strength or reduce the hydraulic conductivity of soil or ground.

Mixing methods (including premixing or deep mixing): Treat the weak soil by mixing it with cement, lime, or other binders in-situ using a mixing machine or before placement

Jet grouting: High speed jets at depth erode the soil and inject grout to form columns or panels.

Compaction grouting: Very stiff, mortar-like grout is injected into discrete zones and remains in a homogenous mass so as to density loose soil.

Compensation grouting: Medium to high viscosity particulate suspensions is injected into the ground between a subsurface excavation and a structure in order to negate or reduce settlement of the structure due to ongoing excavation.

E. Earth reinforcement, this method is further divided in to three categories

Geo-synthetics or mechanically stabilized earth (MSE): Use of the tensile strength of various steel or geo-synthetic materials to enhance the shear strength of soil and stability of roads, foundations, embankments, slopes, or retaining walls.

Ground anchors or soil nails: Use of the tensile strength of embedded nails or anchors to enhance the stability of slopes or retaining walls.

2.3 CHEMICAL AND BIOLOGICAL METHODS OF SOIL STABILIZATION

Soil stabilization is a method of improving soil properties by blending and mixing other materials. Following are the various soil stabilization methods and materials:

Soil Stabilization with Cement: The soil stabilized with cement is known as soil cement. The cementing action is believed to be the result of chemical reactions of cement with siliceous soil during hydration reaction. The important factors affecting the soil-cement are nature of soil content, conditions of mixing, compaction, curing and admixtures used.

Lime, calcium chloride, sodium carbonate, sodium sulphate and fly ash are some of the additives commonly used with cement for cement stabilization of soil.

Soil Stabilization using Lime: Slaked lime is very effective in treating heavy plastic clayey soils. Lime may be used alone or in combination with cement, bitumen or fly ash. Sandy soils can also be stabilized with these combinations. Lime has been mainly used for stabilizing the road bases and the sub-grade.

Lime changes the nature of the adsorbed layer and provides pozzolanic action. Plasticity index of highly plastic soils are reduced by the addition of lime with soil. There is an increase in the optimum water content and a decrease in the maximum compacted density and the strength and durability of soil increases. Normally 2 to 8% of lime may be required for coarse grained soils and 5 to 8% of lime may be required for plastic soils. The amount of fly ash as admixture may vary from 8 to 20% of the weight of the soil.

Soil Stabilization with Bitumen: Asphalts and tars are bituminous materials which are used for stabilization of soil, generally for pavement construction. Bituminous materials when added to a soil, it imparts both cohesion and reduced water absorption. Depending upon the above actions and the nature of soils, bitumen stabilization is classified in following four types:

Sand bitumen stabilization,

Soil Bitumen stabilization,

Water proofed mechanical stabilization, and

Oiled earth.

Chemical Stabilization of Soil: Calcium chloride being hygroscopic and deliquescent is used as a water retentive additive in mechanically stabilized soil bases and surfacing. The vapor pressure gets lowered, surface tension increases and rate of evaporation decreases. The freezing point of pure water gets lowered and it results in prevention or reduction of frost heave. The depressing the electric double layer, the salt reduces the water pick up and thus the loss of strength of fine grained soils. Calcium chloride acts as a soil flocculent and facilitates compaction. Frequent application of calcium chloride may be necessary to make up for the loss of chemical by leaching action. For the salt to be effective, the relative humidity of the atmosphere should be above 30%. Sodium chloride is the other chemical that can be used for this purpose with a stabilizing action similar to that of calcium chloride. Sodium silicate is yet another chemical used for this purpose in combination with other chemicals such as calcium chloride, polymers, chrome lignin, alkyl chlorosilanes, siliconites, amines and quarternary ammonium salts, sodium hexametaphosphate, phosphoric acid combined with a wetting agent.

Electrical Stabilization of Clayey Soils: Electrical stabilization of clayey soils is done by method known as electro-osmosis. This is an expensive method of soil stabilization and is mainly used for drainage of cohesive soils.

Soil Stabilization by Grouting: In this method, stabilizers are introduced by injection into the soil. This method is not useful for clayey soils because of their low permeability. This is a costly method for soil stabilization. This method is suitable for stabilizing buried zones of relatively limited extent. The grouting techniques can be classified as following:

Clay grouting,
Polymer grouting, and
Bituminous grouting

Soil Stabilization by Geo-textiles and Fabrics: Geo-textiles are porous fabrics made of synthetic materials such as polyethylene, polyester, nylons and polyvinyl chloride. Woven, non-woven and grid form varieties of geo-textiles are available. Geo-textiles have a high strength. When properly embedded in soil, it contributes to its stability. It is used in the construction of unpaved roads over soft soils.

Biological Stabilization methods: Use of vegetation roots for stability of slopes.

Microbial Stabilization methods: Use of microbial materials to modify soil to increase its strength or reduce its hydraulic conductivity

2.4 MICROBIALLY INDUCED CALCITE PRECIPITATION (MICP)

Many studies have been conducted to evaluate the strength/ stiffness and permeability of different soils using calcite precipitation induced by microbes. The changes in strength, stiffness,
compressibility and permeability of the treated soil depend on many environmental and other factors that govern the microbial reaction with the required reagents to induce calcite precipitates. Hence, improvement of soil properties is always governed by some physical properties of soil. The degree of saturation of the soil has a considerable impact on the resulting strength and stiffness of the treated soil. It was reported by (Chu & Ivanov, 2012)that particle size distribution, mineralogy, shape, density and texture of the mineral aggregates affect the cementation process in bio-mediated treatment process. However, excellent results demonstrated by this technique in sealing leakages in water retaining structures and reducing the permeability of some soils by means of bioclogging have led to
many interesting researches and applications of biosealing in many civil engineering works (Ivanov & J.chu, 2008). The technique of using microorganisms to improve the strength of granular soil which is referred to as biocementation started in 2001 in Australia.

Construction in microbial biotechnology can be classified in fig 2.1 below

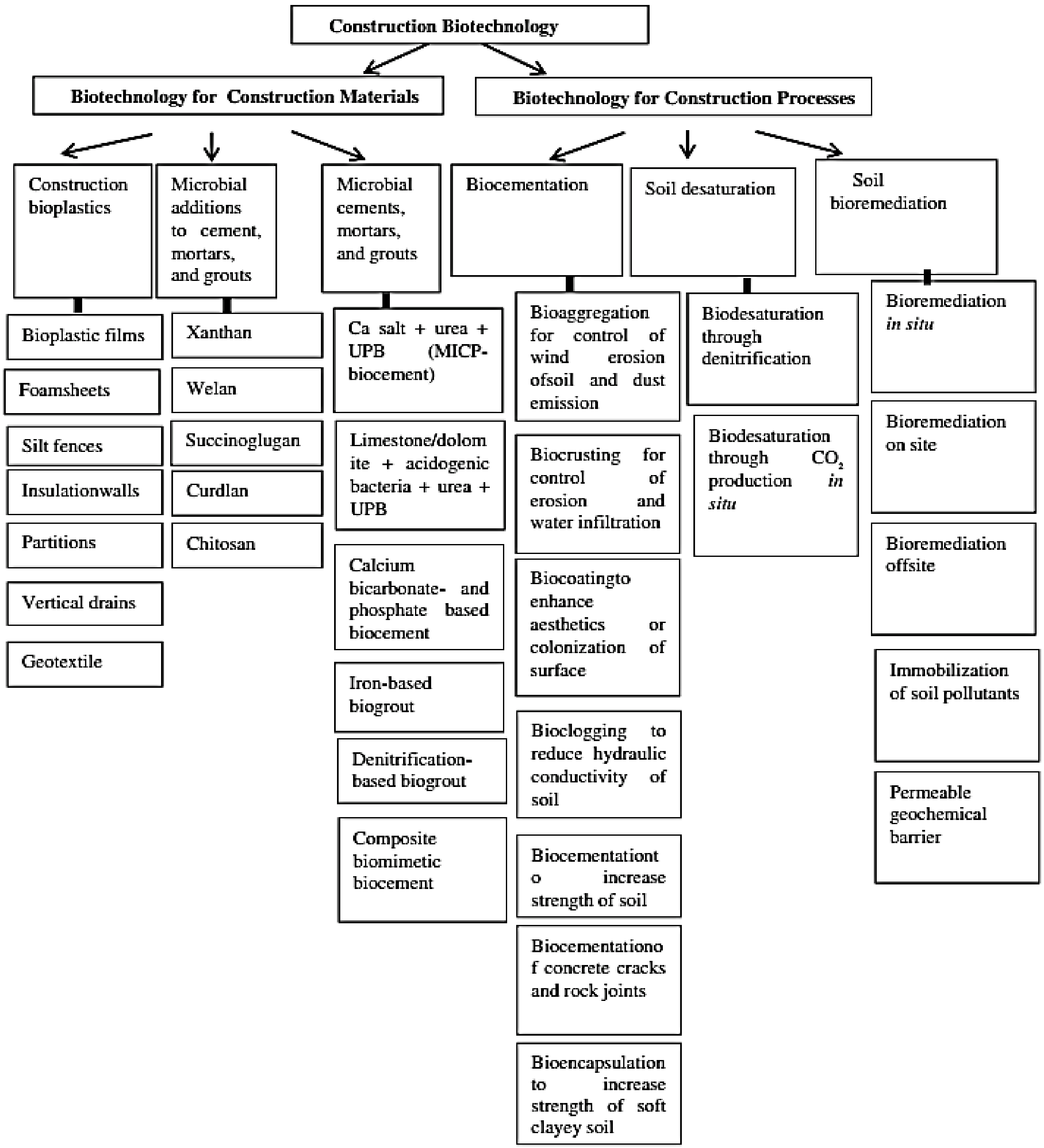

Figure 2-1 Construction in micro biotechnology

Microbial activity provides the opportunity to manipulate soil and improve the soil property using natural or stimulated process. Microbial Geo-technology can be considered as a branch of Geotechnical Engineering aiming to improve the mechanical properties of soil so that it will be more suitable for construction or environmental purposes (Idraratna & chuJ.(Eds), 2005) .

(Ivanov & J.chu, 2008) Reported that interactions between microorganisms and soil or sediment particles can occur in the following ways: microbial production of particle binding material (bio-cementation) and microbial production of pore-filling material (bio-clogging)

Bio-clogging could be used for the following construction and geotechnical applications: 1) to reduce drain channel erosion; 2) for grout curtains to reduce the migration of heavy metals and organic pollutants; 3) prevent piping of earth dams and dikes; 4) construction of aqua-cultural ponds; 5) construction of reservoirs.

Bio-cementation could be used for the following construction and geotechnical applications: 1) to control erosion in coastal area and rivers; 2) construction of aqua-cultural ponds ; 3) construction of reservoirs; 4) construction of dams; 5) to reduce the liquefaction potential of soil; 5) to enhance the stability of slopes and dams; 6) to produce strong filling material from soft soil; 7) soil stabilization in land reclamation; 8) increasing the bearing capacity of foundations; 9) treatment of surfaces to reduce radioactive or toxic dust levels; 10) to increase the resistance of boreholes on oil and gas fields; 11) immobilization of the soil pollutants.

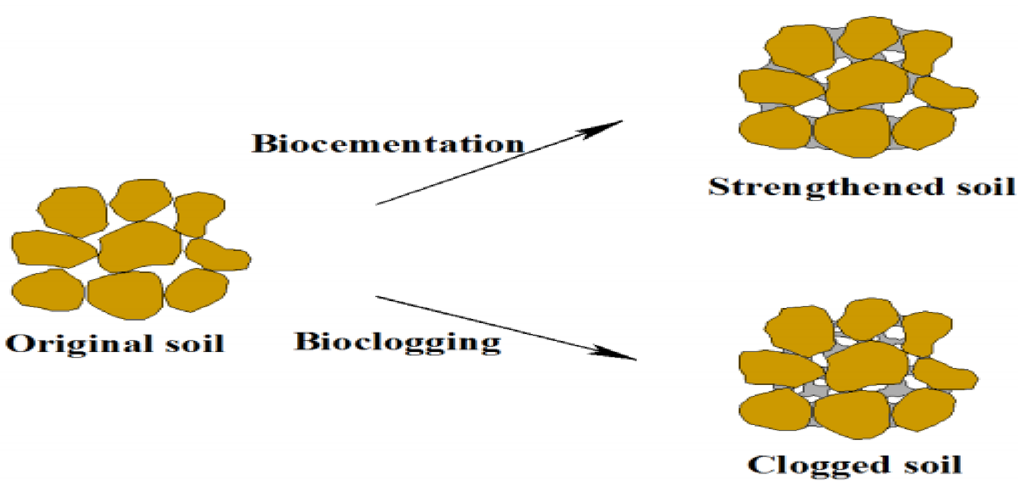

Figure 2-2. Bio cementation and bio clogging

2.4.1 BIO-CEMENTATION

Bio-cementation as defined by (Ivanov & J.chu, 2008) is a process to enhance the strength and stiffness properties of soil and rocks through microbial activity or products. On the other hand, natural cement creation in the earth's crust was studied as chemical deposition and chemical processes with the assistance of weathering. Examples of natural cementing could be seen in treated material such as conglomerate, breccia, sandstone, siltstone, shale, limestone, gypsum (Ivanov & J.chu, 2008), and the formation of ferric hydroxide in the pores of sand. Silica, hydrous silicates, and hydrous iron oxides were mentioned as the facilitated agents in the cementation of sand.

2.4.1.1 MICROBIAL-INDUCED CALCIUM CARBONATE PRECIPITATION (MICP) PROCESS

Calcite precipitation can be induced by several MICP processes. The main categories of the calcite precipitation process include hydrolysis of urea, photosynthesis and sulfate reduction inducing dolomite precipitation (Warthmann, R., Lit, Vasconcelos, & Karpoff, 2000).

Photosynthesis could promote carbonate precipitation with the aid of fungi, algae, and other biogeochemical agents (Ehrlich, 2002).The calcification is the result of calcareous plants, autotrophic nutrient acquisition physiologies in nutrient-deficient environments. For sulfate reduction, the dissolution of gypsum and removal of sulfate by sulfate-reducing bacteria could eliminate the inhibitors for carbonate formation. These activities increase the resultant pH and offer mechanisms that favor dolomite precipitation. A nitrogen cycle involves ammonification, nitrate reduction, or urea hydrolysis. These three mechanisms are all capable of producing calcium carbonate with the same by-products, i.e. ammonia and carbon dioxide (Castanier., Orial, & J.Perthuisot, 1999).

(Van Passen et al, 2006) compared the potential process and concluded that calcite conversion by a urease production process was significantly higher than the other approaches such as aerobic oxidation, sulfate reduction, and denitrification. This is supported by the fact that this study was investigated using the mechanisms of MICP and its applications.

(Ivanov & J.chu, 2008) Urea is hydrolyzed according to Reactions 2.1 to 2.3. Generation of ammonia and carbonic acid is the result of decomposition of carbonate ions (Reaction 2.1). Equilibrium of carbonic acid and ammonia molecules in water leads to an increase in the pH environment (Reactions 2.2 and 2.3) which endorses the precipitation of calcite.

2-1

	$NH_2 - CO - NH_2 + 2H_2O$ $2NH_4^+ + CO_3^2$	**(2-2)**
	H_2CO_3 $HCO_3 + H^+$	**(2-3)**

$$NH_2 - CO - NH_2 + 2H_2O \longrightarrow 2NH_4^+ + CO_3^{2-} \qquad (2.1)$$

$$H_2CO_3 \longleftrightarrow HCO_3 + H^+ \qquad (2.2)$$

$$2NH_3 + 2H_2O \longrightarrow NH_4^+ + 2OH \qquad (2.3)$$

The aerobic bacteria are preferable as they release CO_2 from cell respiration, and CO_2 production is paralleled by the pH rise due to ammonium production. The species of Sporosarcina (previously Bacillus) pasteurii was identified as common alkaliphilic aerobic soil bacteria with high urease activity.

2.4.1.2 MECHANISMS OF BIOCEMENTATION

The bio-cementation process is catalyzed by bacteria or their enzymes. (Dejong & K.nusslein, 2006) Studied controlling ureolysis by aerobic microbes to raise the pH in a super saturated solution, forcing precipitation of calcite within granular porous media. Furthermore, they have measured both increased stiffness, using S-wave velocity measurements, and increased un-drained strength. Various microbiological processes. i) Precipitation, mineral transformation, biofilm and biopolymer growth to attain beneficial changes in engineering properties of soils in short time frames, ii) carbonate precipitation, microbial transformation of smectite to illite, and biopolymer plugging. Conducted research on survivability to accommodate grain size and depth of burial, clogging for the controlled modification of hydraulic conductivity, and gas generation for bulk stiffness control.

Creating uniform cementation is essential for bio-mediated soil improvement to be used for civil infrastructure applications (Dejong.J, D.Nelson, & Y.Fujita, 2009) When microbes are injected into the subsurface, the microbial cells are filtered by the soil matrix. Filtration of cells generally results in a log-linear reduction of microbe concentration along the injection path. The degree of cementation corresponds directly to the stiffness of the soil; therefore, a cementation gradient results in a stiffness gradient. Laboratory results indicate that a push-pull injection process (Dejong.J, D.Nelson, & Y.Fujita, 2009) may effectively counteract the gradient of microbial concentration, resulting in more uniform cementation.

Using a grid of injection/extraction wells, the microbes and nutrients are injected through the soil by inducing an artificial hydraulic gradient. Subsequently, nutrients are injected using a reversed artificial hydraulic gradient. The reversal of injection direction provides more nutrients for consumption by the smaller microbe concentration, and fewer nutrients for the large microbe concentration near the initial injection source. This process improves spatial distribution of the treatment, and, therefore, cementation uniformity across the treated zone.

Investigation of the nucleation site of cells for crystallization of calcite (Dejong & K.nusslein, 2006) shows the balanced Reactions 2.4 to 2.7 for calcite precipitation

$$NH_2 - CO - NH_2 + 3H_2O \longrightarrow 2NH_4^{+} + 2OH^{-} + CO_2 \qquad (2.4)$$

$$Ca^{2+} + CO_3^{2-} \longleftrightarrow CaCO_3\downarrow \qquad (2.5)$$

$$Ca^{2+} + HCO_3^{-} + OH^{-} \longrightarrow CaCO_3\downarrow + H_2O \qquad (2.6)$$

$$Ca^{2+} + 2HCO_3^{-} \longleftrightarrow CaCO_3\downarrow + CO_2 + H_2O \qquad (2.7)$$

Moreover, precipitation of calcite on the cell surface as a consequence of deposition of calcium ions on the surface of negatively charged cells, are shown in Reactions 2.8 and 2.9.

$$Ca_2^{+} + Cell \longrightarrow Cell\text{-}Ca_2^{+} \qquad (2.8)$$

$$Cell - Ca_2^{+} + CO_3^{2-} \longrightarrow Cell\text{- } CaCO_3\downarrow \qquad (2.9)$$

(DeJong, 2010) explained the mechanism of strength improvement contributed by calcite precipitated. The calcite precipitation results in a decrease in void space (porosity), and subsequently provides perception into a change in overall properties. Beyond that, the distribution of calcite within the void space of soil (mm scale) is critical. Figure 2.3 provides schematics of the two extreme possibilities of how calcite may be dispersed around soil particles. (DeJong, 2010) "Uniform" distribution indicates the calcite precipitated on the surface of soil particles evenly, at an equal thickness. As a result, the bonding formed by calcite to cohere two particles is relatively small, and consequently negligible improvement to soil properties may be anticipated. "Preferential" distribution refers to a condition in which the calcite only precipitated at particle-particle contacts. This is the preferred spatial distribution as all calcite precipitated contributes directly to the enhancement in soil properties. Unfortunately, bio-geo-chemical processes do not naturally optimize for soil engineering properties. For that reason the "preferential" distribution is impracticable. Auspiciously, the "uniform" distribution is also not viable. Both of the analysis of scanning electron microscope, SEM (Figure 2.4) and X-ray computed tomography images demonstrate that the balance of these two extreme conditions is the "actual" distribution of precipitated calcite (Figure 2.3) (DeJong, 2010)

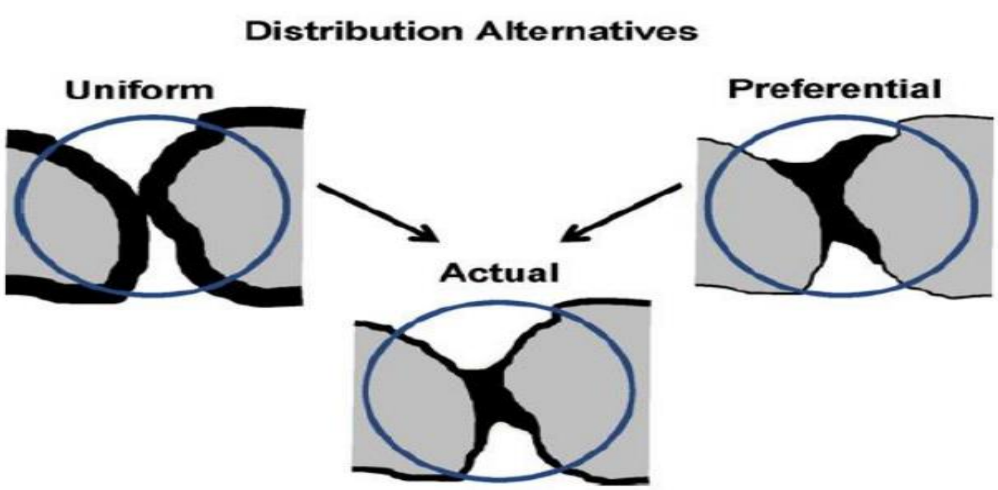

Figure 2-3 Alternative Soil distribution

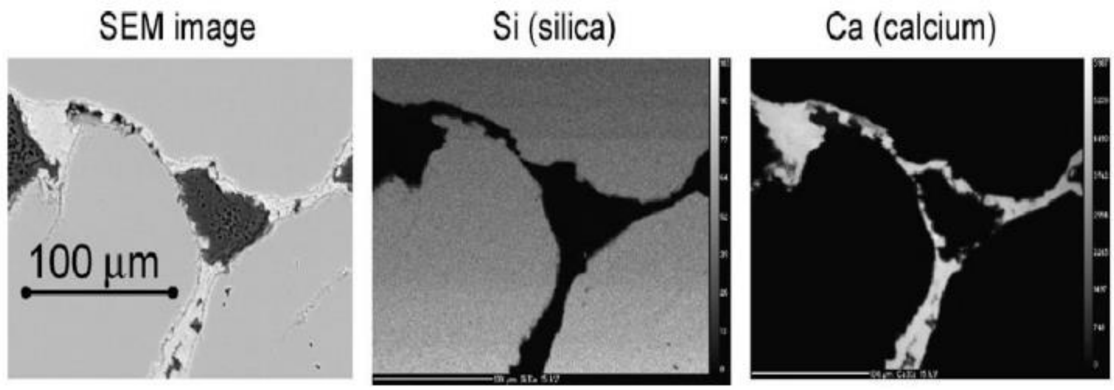

Figure 2-4 SEM and X-ray computed soil particle

Fortunately for the MICP process, there is a considerable fraction of the calcite is in the neighborhood of the particle–particle contacts. The formation of calcite in the soil pore space can be clearly seen in Figure 2.4. The spatial distribution of calcite is determined by biological behavior and filtering processes. Microbes have an inclination to keep away from exposed particle surfaces and instead desire to locate themselves in smaller surface features, such as near particle contacts. This partiality is due to reduced shear stresses in the area and a greater availability of nutrients at the soil grain contacts. A larger concentration of microbes near the particle-particle contacts promotes greater portion of calcite precipitation in that region (DeJong, 2010).

Besides MICP, there are some other potential microbial processes that can lead to bio-cementation as summarized in Table 2.1 (Ivanov & J.chu, 2008). These processes include binding of soil particles with sulphides of metals produced by sulphate-reducing bacteria; carbonates of metals produced due to hydrolysis of urea; and production of ferrous solution, ferric salts and hydroxides due to activities of iron-reducing bacteria.

Table 2-1.Potential microbial processes that can lead to bio-cementation

Physiological group of microorganisms	Mechanism of bio-cementation	Essential conditions for bio-cementation	Potential geotechnical applications
Sulphate reducing bacteria	Production of undissolved sulphides of metals	Anaerobic conditions; presence of sulphate and carbon source in soil	Enhance stability for slopes and dams
Ammonifying bacteria	Formation of undissolved carbonates of metals in soil due to increase of pH and release of CO	Presence of urea and dissolved metal salt	Mitigate liquefaction potential of sand. Enhance stability for retaining walls, embankments, and dams. Increase bearing capacity of foundations.
Iron-reducing bacteria	Production of ferrous solution and precipitation of undissolved ferrous and ferric salts and hydroxides in soil	Anaerobic conditions changed for aerobic conditions; presence of ferric minerals	Densify soil on reclaimed land sites and prevent soil avalanching. Reduce liquefaction potential of soil

2.4.2 BIOCLOGGING

Bio-clogging is a process where the soil void is filled by the product from microbial-induced biochemical process. The clogging of soil restricts water flow through soil, and hence reduces its hydraulic conductivity. (Vandevivere & Baveye.p, 1992) Found that the hydraulic conductivity of soil reduced significantly through the accumulation of biomass and production of exopolymeric substances. The accumulation can occur at soil pore throat or uniformly on soil particle surface. The reduction in hydraulic conductivity induced by the accumulation of biomass in soil matrix is not permanent.

Different possible microbial processes that may lead to bioclogging are summarized in Table 2.2. These processes include a formation of impermeable layer brought by algal and cyanobacterial biomass; slime in soil induced by aerobic and facultative anaerobic heterotrophic bacteria, oligotrophic microaerophilic bacteria and nitrifying bacteria; production of undissolved sulphides of metals by sulphatereducing bacteria. In addition, ammonifying bacteria induces formation of undissolved carbonates of metals. However, not all of these processes have been tested in laboratory and field (Ivanov & J.chu, 2008).

Table 2-2 Possible Microbial process that may lead to bioclogging

Physiological group of microorganisms	**Mechanism of bioclogging**	**Essential conditions for bioclogging**	**Potential geotechnical applications**
Algae and cyanobacteria	Formation of impermeable layer of biomass	Light penetration and presence of nutrients	Reduce of water infiltration into slopes and control seepage
Aerobic and facultative anaerobic heterotrophic slime-producing bacteria	Production of slime in soil	Presence of oxygen and medium with ratio of C:N > 20	Avoid cover for soil erosion control and slope

Oligotrophic microaerophilic bacteria	Production of slime in soil	Low concentration oxygen and medium with low concentration of carbon source	Reduce drain channel erosion and control seepag
Nitrifying bacteria	Production of slime in soil	Presence of ammonium and oxygen in soil	Reduce drain channel
Sulphate-reducing bacteria	Production of undissolved sulphides of metals	Anaerobic conditions; presence of sulphate and carbon source in soil	Form grout curtains to reduce the migration of heavy metals and organic pollutants
Ammonifying bacteria	Formation of undissolved carbonates of metals in soil	Presence of urea and dissolved metal salt	Prevent piping of earth dams and dikes

2.5 FACTORS AFFECTING MICP

Calcite precipitation is a relatively straightforward chemical process regulated mainly by four key elements: (i) calcium concentration; (ii) concentration of dissolved inorganic carbon (DIC); (iii) pH; and (iv) availability of nucleation sites (Castanier., Orial, & J.Perthuisot, 1999)In addition, several environmental parameters such as salinity, temperature, geometric compatibility of bacteria etc. may also govern the performance of calcite precipitation.

2.5.1 PH

Calcite precipitation commences when urea is decomposed by urease enzyme. The urease enzyme is produced by microbial metabolic activities and released to environment. As a result, urea hydrolysis normally occurs around the microbe cell. Like all other enzymes, urease enzyme only active at certain range of pH. With the exception of a small group of acid ureases, microbial ureases generally possess an optimum pH of near neutrality. The urease activity of alkalotolerant bacteria, such as S. pasteurii has an optimum pH value of 8. At pH values below 5, the microbial ureases could be irreversibly denatured .With respect to the relationship between calcite precipitation and pH, numerous studies performed using S. pasteurii found that the MICP reached a plateau at pH values between 8.7 and 9.5 (Mobley & Hausinger, 1995).

2.5.2 BACTERIA CELL CONCENTRATION

A high bacterial cell concentration supplied to the soil sample would certainly increase the amount of calcite precipitated from MICP process The rate of urea hydrolysis has a direct relationship with the bacterial cell concentration, provided sufficient cementation reagent is available. A high concentration of bacteria produces more urease per unit volume to commence the urea hydrolysis (Okwadha & li, 2010). The availability of nucleation sites is one of the key factors for calcite precipitation. (Stocks-Fischer & bang, Microbiological precipitation of CaCo3, 1999) also demonstrated that calcite precipitation is associated with the concentration of Bacillus Pasteurii, one of the urease positive bacteria.

2.5.3 TYPES OF BACTERIA

The bacteria types that are suitable for MICP application should be able to catalyst urea hydrolysis, and they are usually urease positive bacteria. The typical urease positive bacteria are genera Bacillus, Sporosarcina, Spoloactobacilus, Clostridium and Desulfotomaculum (Kucharski, 2008) the aerobic bacteria are preferable as they release CO_2 from cell respiration, and CO_2 production is paralleled by the pH rise due to ammonium production. Bacillus sp. is a more common type of bacteria used to precipitate calcium carbonate in their micro-environment through catalytic conversion of urea to ammonia and carbon dioxide.

2.5.4 NUTRIENTS

Nutrients are the energy sources for bacteria, and hence it is essential to provide proper and sufficient nutrients for urease-producing bacteria. Nutrients are supplied to bacteria during culture stage and soil treatment stage. Common nutrients for bacteria include CO2, N, P, K, Mg, Ca, Fe, etc Lack of organic constituents in soil is a limitation for bacteria growth. The supply of nutrient into soil specimen during soil treatment process is essential. Numerous previous reported studies have included 3 g/l of nutrient broth into the treatment solution to sustain the growth and viability of urease producing bacteria (Dejong & K.nusslein, 2006). The supply of nutrient is to ensure the bacteria can sustain sufficiently long to support calcite precipitation in order to achieve the desired level of improvement.

2.5.5 TEMPERATURE

Temperature has a significant influence on the urease activity, and hence on the rate of MICP. At temperatures below 5^{o}C, the urease activity is negligible (Van Paassen, 2009). (Whiffin, 2004) studied the effect of temperature on urease activity in Sporosarcina pasteurii. He found that the urease activity increased proportionally with temperatures between 25 ^{o}C and 60^{o}C. The enzyme had an optimum temperature of 70^{o}C, after which the urease activity dropped significantly to almost half of the optimum urease activity at 80^{o}C. Despite the urease activity peaks at 70^{o}C, most of the MICP treatments were performed at room temperatures (i.e. 20- 30^{o}C). This is because most of the urease producing bacteria used in the existing MICP treatments (i.e. S. pasteutii, B. megaterium) are mesopilic type with the optimum growth temperatures ranging from 30 - 45^{o}C.

2.5.6 FIXATION AND DISTRIBUTION OF BACTERIA IN SOIL

Ideally, urease positive bacteria should be distributed evenly and fixed in place when they are injected into soil for MICP treatment. Improper method of injection may cause the bacteria to be located only in certain part of soil or be flushed out from the soil (Harkes, 2010) studied on the methodologies to dispense bacteria and settle them over a 18 cm long sand bed. They found that injection of undiluted bacteria suspension, followed by one pore volume of high salinity fixation fluid (50 mm of calcium chloride) could successfully retain almost all bacteria suspension in the sand bed. High salinity solution encourages flocculation, and this promotes the adsorption of bacteria and retention in sand column. Nevertheless, a low salinity solution (e.g. fresh surface water) has its advantage where homogenous distribution of bacteria is required at large sand body. Low ionic strength and adsorption strength of bacteria in the low salinity solution allow them to transport over great distances (Harkes, 2010). Fixation fluids with a high flow rate flush bacteria cell over a longer distance than that of a low flow rate.

2.5.7 INJECTION METHODS

Studies pertaining to the favorable and proper treatment method of MICP can be found in abundance. Most researches on MICP were performed by injection method which is similar to the grouting of artificial material for soil improvement. (Harkes, 2010) Found that two-phase injection procedure could contribute to homogenous distribution of B. pasteurii in sand column. The two-phase injection was by first, injection of B. pasteurii suspensions and second, injection of a fixation fluid (high salt content). This procedure has successfully retained 100% of urease activity in the sand column. The effects of injection methods (stopped-flow injection and continuous injection) on the uniformity of calcite formation in sand column, it is found that stopped-flow injection method (injection of 1.5 pore volume of reagent, followed by 2.5 hours of rest period) offered better uniform cementation. On the other hand, continuous injection method promoted abundant calcite precipitation near the injection point, but the calcite content decreased with the distance from the injection point. The stopped-flow injection is capable of distributing cementation fluid evenly in sand column before the composition of calcite. Repeated injection of reagent or number of treatment to the soil would increase the composition of calcite. The repeated injection of reagent is very similar with the stopped-flow injection.

2.6 COMPARISON OF CONVENTIONAL AND MICROBIAL METHODS

Various applications of MICP have been investigated by researchers which can be categorized in this study according to its application in geotechnical and geo-environmental engineering, other applications such as remediation/stabilization of cracks in concrete, stabilization of dams and embankments. The conventional ground improvement methods that compete with microbial techniques are typically cement-based techniques. The general view of these methods, such as soil stabilization using Portland cement, is that they are harmless/clean in spite of the energy intensive, carbon-producing manufacturing process for cementation materials these methods involve the quarrying of large volumes of raw materials and associated land destruction, coupled with a high pH. Therefore, one key advantage of microbial stabilization methods is their potential for a significant reduction in embodied energy and carbon emissions, relative to cement-based techniques (DeJong J. T., 2012). In addition, microbial methods use natural and biogeochemical processes to improve soil, which makes them non-toxic and environmentally friendly, whereas chemicals are used in the improvement media into the ground, for treatment of the by-products, etc.) Are largely unknown.

In addition, microbial methods – specifically MICP – may enable improvement over larger

distances, owing to their low viscosity and injection pressure requirements. They can be deployed beneath and around existing structures without any disruptions. These general attributes make microbial methods potentially favorable for many ground improvement projects. Therefore, based on related studies and the characteristics mentioned above, microbial soil improvement technologies provide opportunities to address important issues, such as climate change, energy, food, shelter, infrastructure, urbanization, sustainability, waste management, safety, water availability and economic stability, compared to conventional soil improvement methods.

There are also disadvantages associated with microbial stabilization. Microbial processes can sometimes be slower than mechanical and chemical processes. Additionally, environmental issues include pH, temperature, concentration of electron acceptors and donors, concentrations and diffusion rates of nutrients and metabolites should be considered which means that operators must be competent in the technical aspects of microbiology. Subsequently, the construction of conventional methods are relatively easy and don't involve careful monitoring, while the application of microbial methods are complicated and growing conditions should be carefully monitored. Nevertheless, it should be mentioned that the MICP process is not an entirely green technology. During the hydrolysis of urea by UPB through MICP, the by-products (such as ammonia) may cause environmental concerns, such as toxic effects on the health of humans (atmospheric nitrogen deposition), vegetation and aquatic organisms, leading to the eutrophication and acidification of sensitive ecosystems and the discoloration of stones (Camargo, 2005). Furthermore, there has often been public resistance to microbial methods using exogenous organisms. Due to this complexity, the sustainability analyses to date are limited and there are issues that must still be addressed. It is known that soil improvement by the microbial precipitation of calcite requires less carbon than cement stabilization. However, due to the very limited field applications, the actual costs (energy required for manufacturing urea and calcium chloride, for injecting the improvement media into the ground, for treatment of the by-products, etc.) are largely unknown.

Based on recent work by (Chu J. V., 2012) the cost of applying microbial calcite precipitation involves four major parts: enzyme production, expenditure of chemical reagents (urea and CaCl2), waste products treatment and equipment. Enzyme production is the first cost and includes labor, equipment, operation, chemicals, sterilization and transport of the culture from the biotechnology company to the site of use. The cost of chemicals for the cultivation of bacteria is considered one of the major expenses, and economical alternatives are needed for the medium ingredients that account for as much as 60% of the total operating costs.

New findings by (Chu & Ivanov, 2012) have shown that materials and costs could be reduced by more efficient cementation. They discussed the optimal balance of substrates for various applications, which could be used to increase the economic feasibility, reduce the production of unwanted by-products and increase the long term efficacy of these biominerals.

Their cost estimation based on efficient cementation covers a wide range, the lower half of which is competitive with conventional ground improvement techniques such as deep soil mixing, jet grouting and chemical grouting. After considering the general attributes of microbial processes, (DeJong J. e., 2010) identified and evaluated 24 different applications in a qualitative manner using the criteria of cost, implementation, probability of success and social acceptance. The applications and their approximate 'ranking' are summarized and presented in Table 2.3. Note that, due to the very limited field applications, challenges for the actual cost (energy required for manufacturing urea and calcium chloride, injecting the improvement media into the ground and treating the unwanted by-products) are largely unknown. Therefore, the adoption of microbial methods in the industry is expected to take some time.

Table 2-3 Bio meditated soil application and their approximate ranking

Application	**Implementation Easy: 5 Difficult: 1**	**Probability of success High: 5 Low: 1**	**Cost/viability Economics:5 Expensive: 1**	**Societal acceptance High:5 Low: 1**	**Total Scour out of 20Total Scour out of 20**
Structural repair	5	5	3	5	18
Erosion control	4	5	4	5	18

Immobilization of contaminants	5	4	4	5	18
Dust mitigation	4	5	4	5	18
Ground improvement for rural roads	5	5	3	4	17
Shallow carbon sequestration	5	3	4	5	17
Leak management	4	3	4	5	16
Ground improvement for urban road subgrading	5	3	3	4	15
Soil liquefaction mitigation (MICP)	3	5	3	3	14
Ground improvement for ash ponds	1	4	4	5	14
Soil liquefaction mitigation (biogas)	3	3	3	3	12

De-swelling of clays	1	1	1	4	7
Underground creation (tunnel)	1	1	2	1	5
Landfills as new energy resource	3	4	1	2	10
Water storage	3	2	2	2	9

2.7 ADVANTAGE OF BIO MEDITATED SOIL IMPROVEMENT

According to (Dejong J. T., 2008) the advantage of bio meditated soil improvement is listed under.

The development of bio-mediated processes for soil improvement has several characteristics that may prove advantageous relative to industry standard soil improvement techniques. These include:

Reduced costs – use of natural materials, reduced treatment injections, etc.

Reduced impact to the environment – use of natural materials that do not permanently alter subsurface conditions

Improved treatment uniformity – biological processes have potential to enhance spatial uniformity

Optimal treatment concentration – degree of treatment can be controlled and monitored

Adaptable duration – treatments can be removed if only temporary support needed (e.g. by reversal of chemical processes)

Hydraulic and mechanical control – degree of treatment can be adjusted

Flexible implementation – methods can be used in new and retrofit construction

Liquefaction prevention – cementation of subsurface to prevent liquefaction and its damage

Building settlement reduction – reduce settlement and increase bearing capacity for foundations

Dam and levee safety – upstream injection of technique would "plug" erosive piping

Tunneling – soil stabilization prior to tunneling would reduce disruption and increase efficiency

Scour/erosion prevention – treatment would increase resistance to erosive forces of water flow

Bluff and slope stabilization – treatment could provide additional stability needed to prevent failures

Impermeable barriers – barriers to stop/divert subsurface transport of contaminants

Reactive barriers – opportunity for creation of barriers that treat/clean groundwater as it flows

Ground water protection – treatment to immobilize materials before contamination of aquifers

Emergency immobilization – rapidly secure contaminants from hazards (e.g. terrorist activities)

Aquifer storage and recovery – treatment to enhance storage and reduce losses in aquifers

Energy (fuel) storage – used to create subsurface facilities for storage of liquefied natural gas

Carbon sequestration – used to create subsurface facilities for storage of CO2.

3 CHAPTER THREE: MATERIAL AND METHOD

3.1 INTRODUCTION

This chapter aims to evaluate microbial induced calcite precipitation method as a soil stabilization in order to evaluate this method and reach in to conclusion the paper evaluate Three researchers material and data regarding MICP soil stabilization method. The material and data used of researcher 1 (Mujah & Shahin, 2016) , researcher 2 (Jawad & Zheng, 2016) & researcher 3 (Cheng & Ralf Cord-Ruwisch, 2013) will be an input for this paper.

It will evaluate the material and data of the above mentioned researcher study regarding MICP soil stabilization method. By looking at the material & data used in order to test unconfined compressive (UCS) & hydraulic conductivity of a given MICP treated soil sample. The method to analyze the work of these researchers is by preparing a chart in order to reach in to conclusion about the effectiveness of MICP soil stabilization method. All the soil samples will be treated with Bactria sample & bacteria nutrient in order to Create MICP process, which will precipitate calcium carbonate. Calcium carbonate in the soil will help the soil particle to bind together.

The treated soil samples have been treated with different saturation and for different saturation the soil sample were tested for compressive strength & permeability.

3.2 MATERIAL

3.2.1 SOIL MEDIA

Research 1

Natural silica sand obtained from Cook Industrial Minerals Pty. Ltd Western Australia was used in this study. The sand is classified as poorly graded according to the unified soil classification system (USCS) with particle size of 0.425 mm. The particle size distribution of the sand used is shown in Figure 3.1.

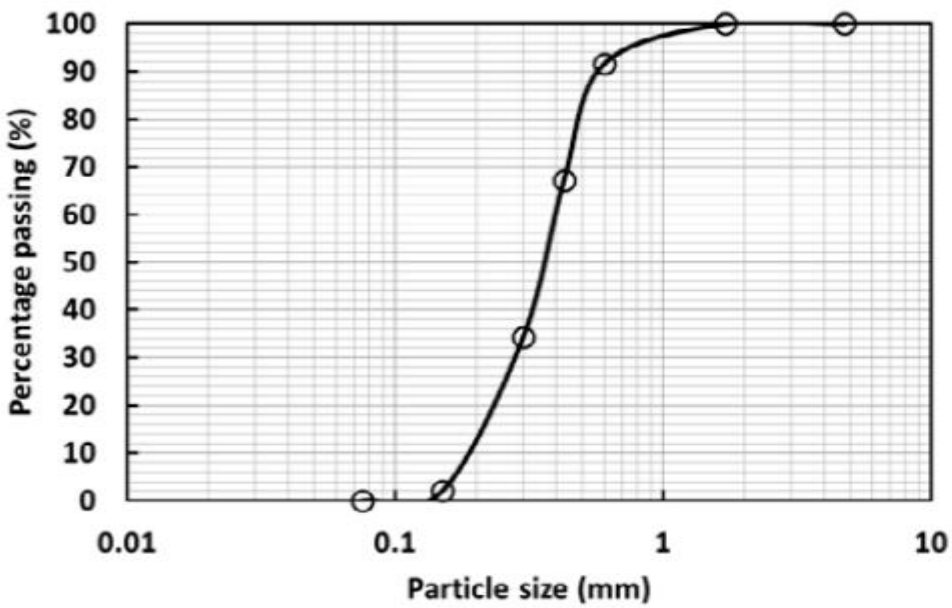

Figure 3-1 Particle size distribution of researcher 1

Resarch 2

Pure silica sand grinded to particle size of 0.1mm was selected for the current study (Fig. 3.2). The sand is classified as poorly graded according to the unified soil classification system (USCS) with particle size of 0.1 mm.

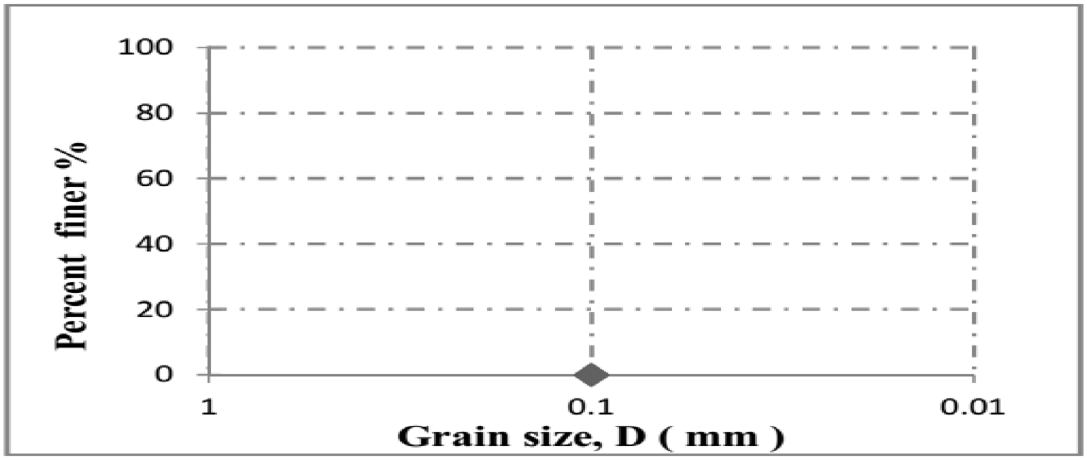

Figure 3-2 Particle size distribution of researcher 2

Research 3

Two different types of pure silica sand (Cook Industrial, Minerals Pty. Ltd., Western Australia) were selected for the current study. Sieve analysis was performed for both fine- and coarse grained sands to determine the particle-size distribution, which is one of the primary components that govern the mechanical behavior of soils. The particle-size distribution curves of the fine and coarse sands used are shown in Fig. 3.3. Both sands are classified as poorly graded sand according to the Unified Soil Classification System (USCS; ASTM 2006). Poorly graded sands were selected as they exhibit undesirable engineering behavior for most geotechnical engineering applications. Both sands have a specific gravity of 2.62.

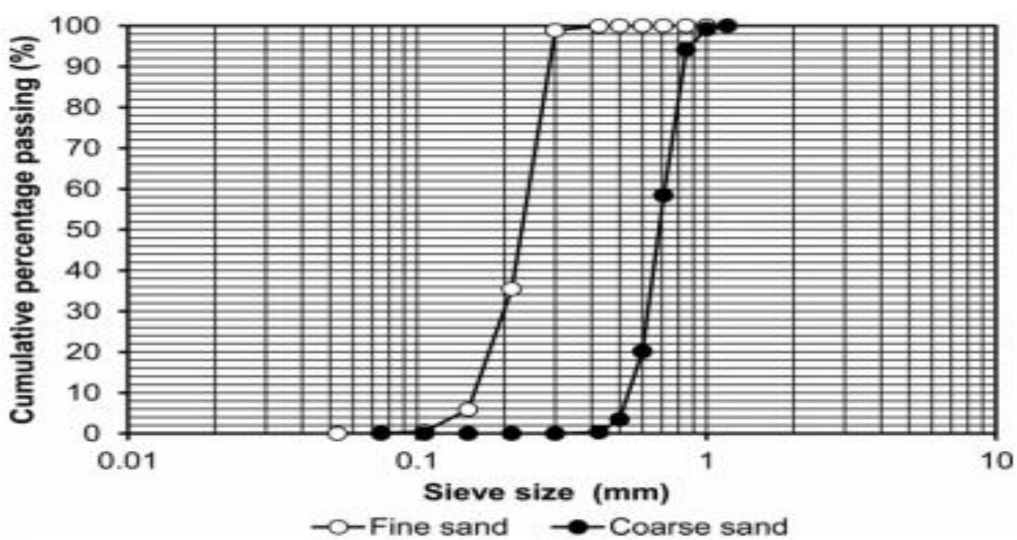

Figure 3-3 Particle size distribution of researcher 3

3.2.2 BACTERIA CULTURE AND CEMENTATION SOLUTION

Research 1

The microorganism used in this study was *Bacillus sphaericus* strain under sterile aerobic condition, the bacteria strain was cultivated in a growth medium consisting of yeast extract and ammonium sulphate.

The PVC mold was up-flushed with 1 void volume of deionised water to remove the air voids inside the sand samples, which were then left to be fully saturated (S = 100%) for 24 hours prior to MICP treatment. The sand column was then down-flushed with 0.5 void volume of bacteria culture, followed by 0.5 void volume of cementation solution. A retention time of 24 hours was adopted for each treatment cycle, to ensure the bacteria attachment to the sand particles.

Research 2

The ureolytic bacterium used in the current study was *Sporosarcina pasteurii* (ATCC 11859). The ATCC 11859 was cultivated under sterile aerobic batch conditions in a yeast extract medium (20 g/l yeast extract, 10 g/l ammonium sulfate, 0.13 M Tris buffer, pH = 9).

Microbially induced carbonate precipitation for soil treatment was conducted using gravity induced downward precipitation at a flow rate of 0.150 l/h. Initially, the sand columns were divided into two groups. The first group was dry sand columns, and the second group was saturated sand columns. The two groups were flushed with 33 ml bacterial culture, followed by 3 hours of retention time and then repeated flushes with a 33 ml cementation solution. The MICP reaction time was 24 h with highly concentrated cementation solution.

Research 3

The urease active strain of *Bacillus sphaericus* (MCP-11) was used in the experiments. The isolated strain (MCP-11) was cultivated under a sterile aerobic batch condition in a yeast extract–based medium (20 g/L yeast extract, 0.17 mol/L ammonium sulfate).

Alternating injection of equal volumes of bacterial suspension and cementation solution with an inflow rate of about 1 L/hour. The total volume of the introduced solutions was the same as the aforementioned water volume so as to keep a constant degree of saturation. A vacuum pump was connected to the bottom of the PVC column to remove the excess solution. Curing for 12 hours at $25 \pm 1^{o}C$ to allow the bacterial fixation process to complete.

3.2.3 UCS TESTS

Research 1

The samples were trimmed into 80 mm in length and 40 mm in diameter to maintain an aspect length-to-diameter ratio of 2. The UCS test was conducted using the GCTS STX-300 fully automated apparatus according to the Australian Standards AS5101.4 (2008). The axial load was applied at a constant speed of 1 mm/min. Termination of the experiment was done when either: (1) clear shear failure plane was observed along the column; or (2) the axial displacement reached 20 mm.

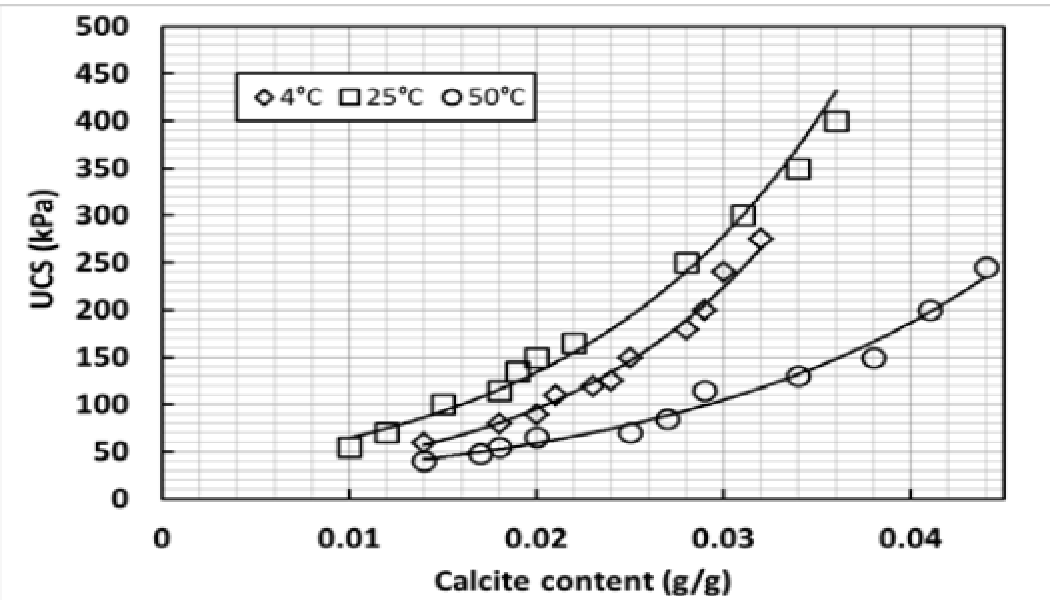

Figure 3-4 Relation between strength and calcite content (Research 1)

Research 2

The unconfined compression test (UCT) was conducted at a constant loading rate of 1.5%/min in accordance with ASTM D2166/D2166M-13 (ASTM 2013). These tests were conducted to establish the relationship between the strength of the soil samples and its CaCO3 content and crystal formation. Before the UCT test, half of the soil samples were washed with 1 L of tap water and then submerged in basin contains tap water for 24 h, followed by an air dried process at 30°C for 24 h.

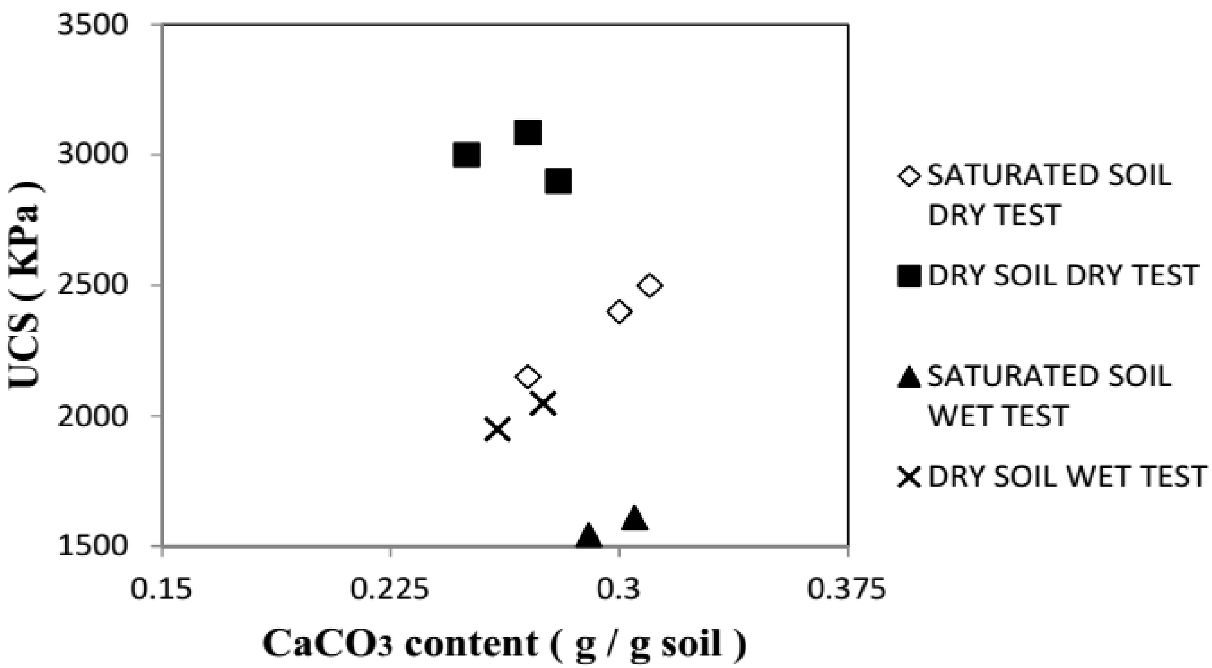

Figure 3-5 Relation between strength and calcite content (Researcher 2)

Research 3

To quantify the strength imparted into the MICP treated silica sand under different saturation conditions, the unconfined compressive strength (UCS) tests were conducted on cemented specimens of 55 mm in diameter with a selected diameter to height ratio of 1:1.5 to 1:2. The axial load was applied at a constant rate of 1.0 mm/min. Before carrying out the tests, the sand samples were treated with different amounts of MICP under 20%, 40%, 80% and 100% degrees of saturation.

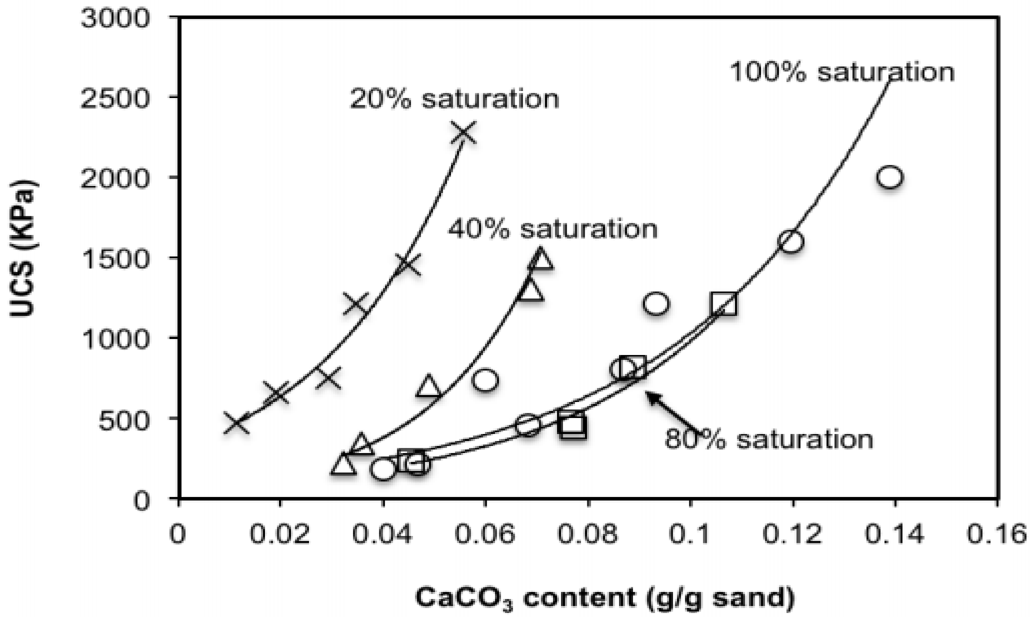

Figure 3-6 Relation between strength and calcite content (Research 3)

3.2.4 PERMEABILITY TEST

Research 1

The permeability measurement in this study was carried out based on the constant head permeability test as outlined in the Australian Standards AS1289.6.7.1 (2001). The reduction in permeability in terms of the percentage of precipitated calcite crystals was determined through the difference in values taken before and after treatment. Permeability is an important factor in soil mechanics as it governs the behavior of porous materials in many geotechnical engineering applications. Figure 3-7 shows the measured permeability values at different cement content of MICP treated soil.

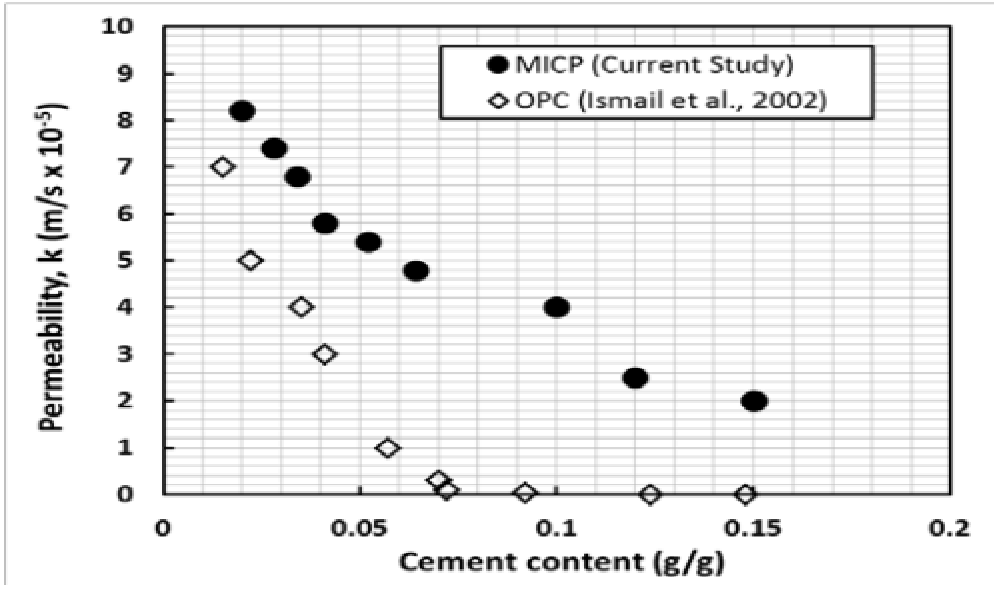

Figure 3-7 Relation between permeability and calcite content (Research 1)

Research 2

The coefficient of permeability of the cured specimens (still contained in molds) was determined using the falling-head permeability test. Treatment cycles using high concentration solution of urea and CaCl2 produced greater reduction in coefficient of permeability of sand. In addition, Fig. 3-8 shows a comparison of measured permeability between saturated and dry treated soils. It can be seen that the initial water content has a significant impact on the effectiveness of bio-clogging. In general, saturated sand samples showed higher calcium carbonate content than the dry soil samples, which is reflected on the permeability values, where the decrease in the permeability of saturated sand samples was slightly greater than that of dry samples. After all, in both cases (saturated and dry samples) the use of MICP technique has significant effect on permeability, where the average decreases in the permeability of the dry and saturated sand samples were approximately 87% and 90%, respectively, after seven treatment cycles.

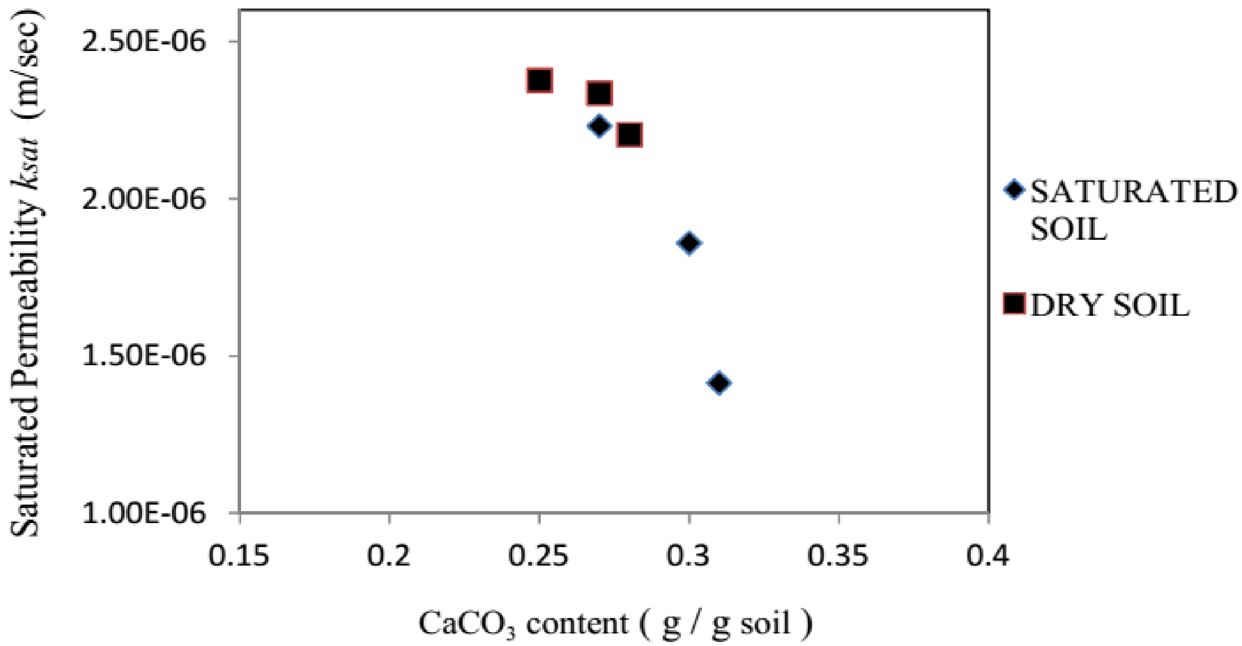

Figure 3-8 Relation between permeability and calcite content (Research 2)

Research 3

Permeability is a primary factor that controls the behavior of porous materials under saturated conditions and thus dictates the suitability of a specific material for certain applications (Shahin et al. 2011). Porous materials with high permeability can prevent the development of excess pore water pressure during loading. To identify the permeability of cemented sand treated with different amounts of CaCO3 precipitates, more samples were prepared at degrees of saturation of 30%, 65% and 100%, and permeability tests were conducted. The permeability test was also conducted on the untreated samples for the purpose of comparison with the treated samples.

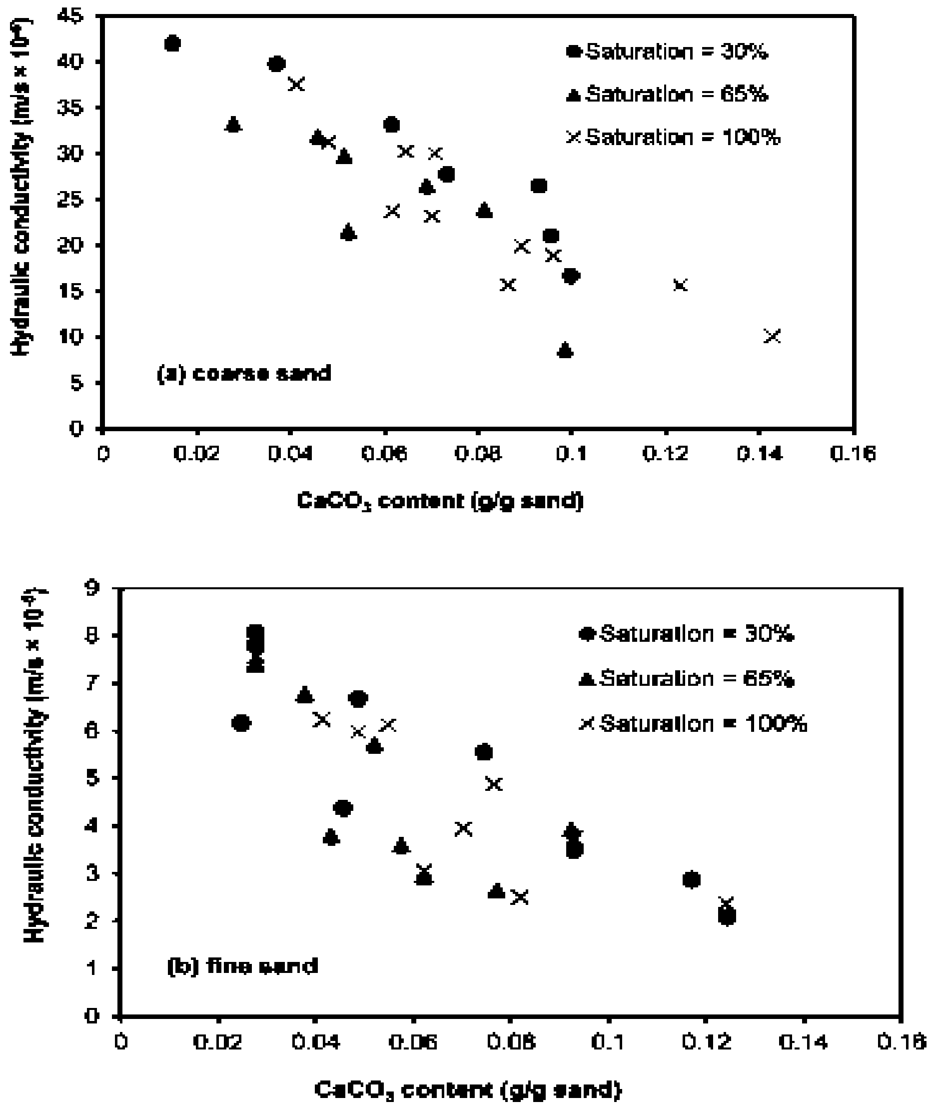

Figure 3-9 Relation between permeability and calcite content (Research 3)

3.3 METHOD OF DATA ANALYSIS

From the above experimental data collected from researcher (Jawad & Zheng, 2016), (Mujah & Shahin, 2016) & (Cheng & Ralf Cord-Ruwisch, 2013) A table is prepared. This contains all researcher results and material used. It contains what kind of sand used, fine or course including the sieve size. It contains what kind of bacteria each researcher used in order to carryout Microbial induced calcite process and the result of each researcher regarding the unconfined compressive strength and permeability of a given sample. The chart will help to compare the effectiveness of MICP under different sand sample and different bacteria content and draw a conclusion depending on the researchers result.

Table 3-1 From Fig 3-4, 3-5 & 3-6 Minimum and maximum Unconfined compression test result regarding to calcite content ($CaCO_3$)

		Calcite content $CaCO_3$	UCS in Kpa
Research 1	Min	0.01	50
	Max	0.04	400
Research 2	Min	0.25	2,200
	Max	0.33	2,500
Research 3	Min	0.04	200
	Max	0.14	2,000

Table 3-2 From Fig 3-7, 3-8 & 3-9 Minimum and maximum permeability test result regarding to calcite content ($CaCO_3$)

		Calcite content $CaCO_3$	Permeability m/s x10^n
Research 1	Min	0.02	8 X 10-5
	Max	0.15	2 X 10-5
Research 2	Min	0.257	2.3 X 10-6
	Max	0.33	1.4 X 10-6
Research 3	Min	0.043	6.3 X 10-5
	Max	0.13	2.5 X 10-5

The tables above are an input to evaluate the effectiveness of microbial induced calcite process in permeability and strength of a treated soil. Comparative chart has been drawn from the tables which evaluate the effectiveness of a microbial treated soil according to strength and permeability.

4 CHAPTER FOUR: RESULT AND DISCUSSION

4.1 INTRODUCTION

This chapter presents evaluated result of three researchers microbial treated soil sample test result for determining the effectiveness of microbial induced calcite process on improving the strength and permeability of a fine grained soil sample.

Different soil samples were treated and it gave different amount of $CaCO_3$ content accordingly the strength and permeability of the soil varies. In the table 3.1 & 3.2 it shows different $CaCO_3$ content range from 0.01 to 0.33 have a different effect on the strength and permeability. Microbial induced calcite precipitation method uses a bacteria and urea as the bacteria food source in which process calcium carbonate is produced this $CaCO_3$ will bind the soil particle and improves the engineering property of the soil like the strength and permeability. There are different kinds of soil improvement methods some of them are coasty, ineffective, time consuming and environmentally unfriendly according to different researcher like Jawad, Zheng, Mujah, Shahin, Cheng & Ralf Cord-Ruwisch microbial induced calcite precipitation method is environmentally friendly, effective especially for sand soil and reduces cost if properly executed. This paper tries to evaluate the effectiveness of this method for improving the engineering property of the soil in which it uses three researchers (Mujah & Shahin, 2016), (Jawad & Zheng, 2016), (Cheng & Ralf Cord-Ruwisch, 2013) test result as an input, this chapter will support the evaluation by using a chart in order to identify how much calcium carbonate content will improve the soil, draw a conclusion on the relationship between $CaCO_3$ content in the soil improvement in strength and permeability.

Unconfined compression test

Unconfined compression tests are conducted to examine the effects of the improvement exerted on the stiffness and the strength of treated sand samples. Three researcher results have been taken for evaluation all the researchers used microbial induced method to treat the soil. The amount of $CaCO_3$ content in the soil has a great impact on the strength of the soil in which it is shown in the chart below.

Permeability test

This test method covers the determination of the coefficient of permeability of a soil under investigation. The void ratio of a soil has an important effect on permeability. The three researchers used microbial induced method to improve the permeability of the soil sample as shown in the chart below. The microbial induced method help to bind the soil grains together that way it decreases the permeability. The MICP method will produce $CaCO_3$ this calcium carbonate content will crystalize the soil this way the $CaCO_3$ content have a great role on improving the soil sample.

4.2 RESULT

Microbial induced calcite precipitation has been shown to be an effective method to enhance the shear strength and reduce hydraulic conductivity of soil. The soil with enhanced strength can contribute to a greater ground bearing capacity, while reduced hydraulic conductivity can minimize settlement, shrink-swell tendency, seepage, and infiltration of rainfall into soils.

4.2.1 IMPROVED SOIL STRENGTH

From the three researchers unconfined compressive strength test result, table 3.1 has been drawn out in chapter three. All the researchers have found an improvement in the strength of the soil in different calcium carbonate content ($CaCO_3$). In the table 3.1 maximum and minimum $CaCO_3$ and unconfined compressive test result were taken in order to evaluate the impact of microbial treated soil which is directly related to $CaCO_3$ content in a given soil sample in order to have a uniform $CaCO_3$ the data collected in chapter three (table 3.1) has been interpreted by interpolation in to a linear figure 0.05,0.1,0.15,0.2,0.25,0.3 & 0.35, this $CaCO_3$ content is taken in order to have a common ground between the three researcher data which have a great impact on the evaluation process.

Table 4-1 Relationship between UCS & $CaCO_3$ content

$CaCO_{3\ in}$ g/g	UCS in Kpa
0.05	560.00
0.1	1,360.00
0.15	2,018.18
0.2	2,109.09
0.25	2,200.00
0.3	2,387.50
0.35	2,575.00

Using the table above a graph have been plotted, the graph shows the relationship between UCS and $CaCO_3$.

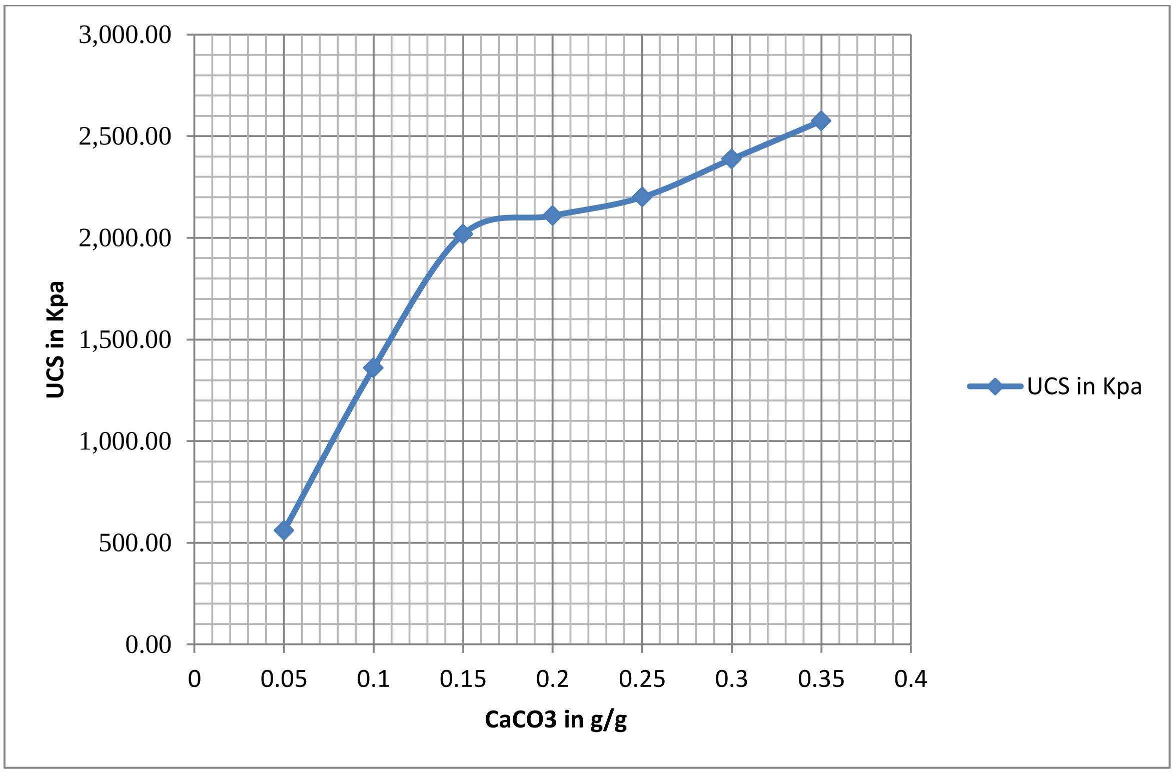

Figure 4-1 Relationship between UCS and $CaCO_3$

4.2.2 IMPROVED SOIL PERMEABILITY

From the three researchers permeability test result, table 3.2 has been drawn out in chapter three. All the researchers have found an improvement in the hydraulic conductivity of the soil in different calcium carbonate content ($CaCO_3$). In order to evaluate the permeability of a microbial treated soil sample the research data taken from three researchers maximum and minimum $CaCO_3$ and permeability. The data collected in chapter three (table 3.1) has been interpreted by interpolation in to a linear figure 0.05,0.1,0.15,0.2,0.25,0.3 & 0.35. From the data collected in chapter 3 a relationship between permeability and $CaCO_3$ has been drawn as shown in table 4.2, which plays a great role in the evaluation process.

Table 4-2 Relationship between Permeability & $CaCO_3$ content

$CaCO_3$ in g/g	Permeability m/s $x10^n$
0.05	5.99425E-05
0.1	3.81034E-05
0.15	0.00002
0.2	1.1729E-05
0.25	3.45794E-06
0.3	1.76986E-06
0.35	1.15342E-06

Using the table above a graph have been plotted, the graph shows the relationship between permeability and $CaCO_3$.

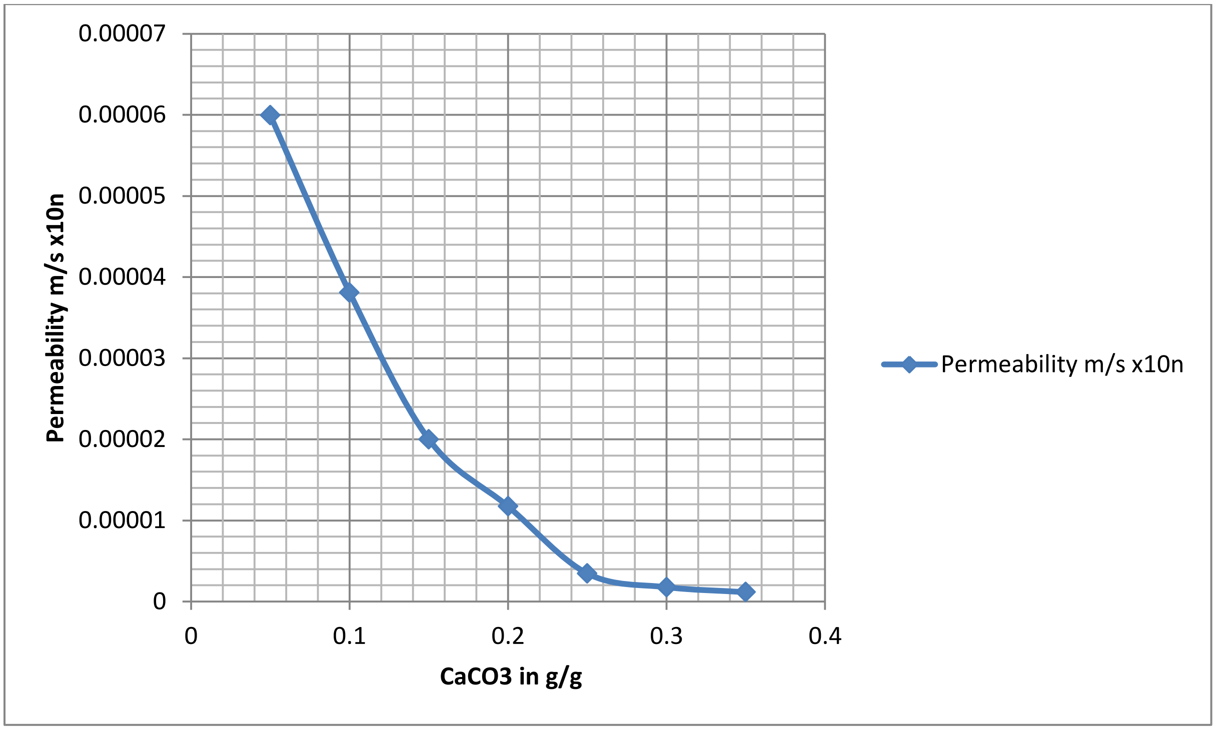

Figure 4-2 Relationship between Permeability and $CaCO_3$

4.3 DISCUSSION

Microbial induced calcite precipitation has been shown to be an effective method to enhance the shear strength and reduce hydraulic conductivity of soil. The soil with enhanced strength can contribute to a greater ground bearing capacity, while reduced hydraulic conductivity can minimize settlement, seepage and infiltration of rainfall into soils.

The experimental results indicated that MICP was more effective in improving shear strength and hydraulic conductivity of a given soil, when the amount of calcium carbonate ($CaCO_3$) is increased. figure 4.1 shows that the unconfined compressive strength of the soil is increased with an increased amount of $CaCO_3$, when the amount of $CaCO_3$ content is 0.05g/g the value of Unconfined compression test result of the soil is 560Kpa and when the amount of $CaCO_3$ content is 0.35g/g the value of the unconfined compression test result of the soil is 2575 Kpa. The increment of UCS test result value is due to the increment in calcium carbonate, in order for MICP test result to be effective in improving the strength of the soil the amount of $CaCO_3$ concentration in the soil plays a great role. The same thing works for improving the permeability of the soil as shown in figure 4.2 when the amount of $CaCO_3$ content is 0.05g/g the value of permeability test result is 5.99425 X 10^{-5} m/s and when the value of $CaCO_3$ content is 0.35g/g the value of permeability test result is 1.15342 X10^{-6} m/s. The reduction in permeability of soil is increased with an increased $CaCO_3$ content. This observation of improved strength and permeability depends on the amount of $CaCO_3$ concentration.

Six different calcium carbonate amount have been recorded starting from 0.05g/g up to 0.35g/g the more the amount of $CaCO_3$ the best effect in improving permeability and strength. For microbial induced calcite precipitation process effectiveness the amount of calcium carbonate concentration plays a great role.

5 CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 SUMMARY AND CONCLUSIONS

MICP is currently of particular interest to engineering and microbiologists. The technique involves introducing aerobically cultivated bacteria with highly active urease enzyme in to the soil harnessing the urease enzyme to catalyze the hydrolysis of urea to produce ammonium and carbonate ions which will cristalize & bind the soil particles hence altering the soil characteristics and increase the shear strength & stiffness, while maintaining adequate permeability therefore it is considered as an alternate & green soil stabilization method.

The promising applications of MICP are Bio-clogging and bio-cementation which could be used to improve mechanical properties of soil in situ. Bio-clogging is the production of pour filling materials between the soil grains while bio-cementation is the production of pour binding material. They can replace energy demanding, expensive and environmentally unfriendly methods with significant reduction in cost. Special attention has to be given for the factors that affect this process.

MICP stabilization method as showed in chapter four it will increase the soil strength and also reduce the permeability of a given soil particles based on the amount of calcium carbonate content concentration in the soil. Since the working capacity of MICP soil stablization technique is strongly depends on $CaCO_3$ it is strongly recommended to give a grate attention on the $CaCO_3$ precipitation.

Most suitable microorganisms for large - scale construction and environmental problems are facultative anaerobic and microaerophilic bacteria. However, industrial - scale applications of microorganisms in geotechnical engineering are yet to be determined.

MICP method has been approved to be a viable alternative for engineering soil improvement applications such as soil embankments, liquefiable sand deposits and subgrade reinforcement.

5.2 RECOMMENDATIONS

1. The study of this project gave an insight about MICP soil stabilization method effectiveness on improving the shear strength and permeability of a given soil sample but it needs a detailed study on to what extent it improves the shear strength and permeability of the soil sample.

2. To further investigate the detail working mechanism of MICP process both on the bio chemical process and the Engineering technique of this method in adapting to our country.

3. To create awareness for the construction industry to use this method in order to adopt an eco-friendly construction method.

4. To further study about the practical approach of this method in Ethiopia.

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